IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application Number	: 10/580,248	Confirmation No.:	6084
Applicant	: Mimi ADACHI, *et al.*		
35 U.S.C. § 371 Date	: July 20, 2006		
Title	: METHOD FOR PROLIFERATING CARDIOMYOCYTES		
TC/Art Unit	: 1632		
Examiner:	: Magdalene K. Sgagias		
Docket No.	: 64517.000003		
Customer No.	: 21967		

Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132

Sir,

I, Hiromitsu Takagi, Ph.D., declare that:

1) I received a Bachelor of Science degree in Pharmaceutical sciences from Tohoku University, Miyagi, Japan, in 1990 and a Doctor of Philosophy degree in Pharmaceutical sciences from the Tokyo University of Science, Chiba, Japan, in 2011.

2) I have been employed by Asubio Pharma Co., Ltd. (or its predecessor),[1] the Assignee of above-named U.S. Application No. 10/580,248 ("the '248 application"), since 1992 as a researcher, and I have been working as a manager and senior researcher since 2007. I was also a research fellow of University of Medicine and Dentistry of New Jersey – New Jersey Medical School from 2005 to 2007.

3) Through the period my research activity, I have worked in the field of cardiovascular research, pharmacology, cell biology, regenerative biology and gene therapy research for

[1] Daiichi Sankyo Co., Ltd. was previously named Asubio Pharma Co., Ltd., which was previously named Daiichi Asubio Pharma Co., Ltd. *See* Reel/Frame Nos. 025025/0335; 019226/0625; and 018115/0304.

approximately 20 years and have studied the cell cycle control and proliferation mechanism of cardiomyocytes during this period.

4) Based on my academic training and professional experience, I consider myself to be at minimum a person of ordinary skill in the technology of regenerative biology, gene therapy research and cardiovascular research, and I was such a person prior to November 21, 2003, the priority date claimed in the '248 application.

5) I am a named inventor of the '248 application. I have been asked to comment on the results in Examples 4 and 5 of the '248 application. The experiments described in these examples were performed either by me personally or under my direction and control.

6) Example 4 teaches the introduction of (1) a cyclin D and a CDK4 gene; (2) a cyclin gene, a CDK4 gene, and a Skp2 gene; (3) a Skp2 gene alone; and (4) a control vector into cardiomyocytes. Cell numbers were counted as a measure of cardiomyocyte proliferation. The results are described in Example 4 and Figure 8 and can be represented as follows:

Group	Increase in Proliferation (observed after 7 days of culture)	Increase in Group Compared to Control (Group - Control) = increase in proliferation (folds)
Control	1.8	0
Skp2	2.0	.2
D1+CDK4	3.2	1.4
D1+CDK4+Skp2	5.3	3.5

7) These results show that when (1) a cyclin D and CDK4 gene; and (2) Skp2 gene alone are introduced into a cardiomyocyte, a 1.4 fold and .2 fold increase in proliferation are observed compared to a control, respectively. Using these numbers, one of ordinary skill in the art would have expected a 1.6 fold increase of proliferation if a cyclin D, CDK4 gene, and Skp2 gene were introduced into cardiomyocytes compared to the control.

8) However, when a cyclin gene, a CDK4 gene, and a Skp2 gene were introduced into a cardiomyocyte, a 3.5 fold increase in proliferation was observed. This significant increase in proliferation was surprising and unexpected.

9) A similar result was observed in Example 5. Example 5 teaches the introduction of (1) a cyclin D and a CDK4 gene; (2) a cyclin gene, a CDK4 gene, and a siRNA specific to the $p27^{Kip1}$ gene ("p27 siRNA"); (3) a p27 siRNA alone; and (4) a LacZ expression virus (control vector) into cardiomyocytes. Cell numbers were counted as a measure of cardiomyocyte proliferation. The results are described in Example 5 and Figure 10 and can be represented as follows:

Group	**Increase in Proliferation (observed after 7 days of culture)**	**Increase in Group Compared to Control (Group - Control) = increase in proliferation (folds)**
Control	1.2	0
Skp2	1.3	.1
D1+CDK4	2.8	1.6
D1+CDK4+ p27 siRNA	4.6	**3.3**

10) These results show that when (1) a cyclin D and CDK4 gene; and (2) p27 siRNA alone are introduced into a cardiomyocyte, a 1.6 fold and .1 fold increase in proliferation are observed compared to a control, respectively. Using these numbers, one of ordinary skill in the art would have expected a 1.7 fold increase of proliferation if a cyclin D, CDK4 gene, and p27 siRNA were introduced into cardiomyocytes compared to the control.

11) However, when a cyclin gene, a CDK4 gene, and a p27 siRNA were introduced into a cardiomyocyte, a 3.3 fold increase in proliferation was observed. This significant increase in proliferation was surprising and unexpected.

I declare that all statements made herein are based on personal knowledge or upon information and belief and are believed to be true; and further that the statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above-identified application or any patent issuing thereon.

Dated: February 16, 2012

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